

OVODAN BIOTECH A/S Havnegade 36 · DK-5000 Odense C

T +45 6611 1732 E: customerservice@ovodanbiotech.com www.ovodanbiotech.com

VAT-no: DK 33 87 02 56

# **Product Datasheet**

Spike-RBD 319-514\_HIS recombinant protein

# For research use only

Description:	Spike-RBD 319-514_HIS   Expressed in HEK-cell Expi293F system. Protein carries a poly-his tag at the N-terminus.   Correct sequence confirmed by Mass Spectrometry, where full coverage of the sequence has been obtained.   Spike-sRBD 319-514   His-tag				
	Calculated MW: 27 kDa Protein migrates as appox. 37 kDa due to glycosylations (See SDS-page beside). Glycan structures are confirmed, and glycosylation sites identified by Mass Spectrometry of protein samples with and without PNGaseF treatment. (see detailed results below). Identified glycosylation sites: N42(IT) and N54(AT). Glycan structures have a combined mass of approx. 6 kDa. Dimerization percentage < 10%	1,5µg RBD	1 µg RBD	111111111	250 130 100 70 55 35 25 15
Formulation:	In PBS solution pH=7.4				

Purification: Immobilized metal affinity chromatography, NiNTA.

Purity: > 95% as determined by SDS-PAGE

**Storage:** Store at -70°C short term. Avoid freeze thaw cycles.





### Bioactivity: ELISA: Specificity towards Human ACE2

Immobilized Human ACE2 1  $\mu$ g/mL (100 $\mu$ L/well) can bind SARS-CoV-2 Spike RBD 319-514 recombinant protein in a sandwich ELISA setup: ACE2 receptor + RBD recombinant antigen + anti SARS-CoV-2 Spike RBD 319-514 polyclonal IgY + Rabbit anti chicken IgG-HRP.



## ELISA: High immunogenicity verified by immunization of hens.

IgY shows strong antigenicity. Immobilized SARS-CoV-2 Spike RBD 319-514 recombinant protein at 1  $\mu$ g/mL (100 $\mu$ L/well) binds chicken anti- SARS-CoV-2 Spike RBD 319-514 with a linear range between 64 to 256 ng/mL antibody added over fixed antigen concertation coated on the well. Starting concentration of antibody normalized to 1  $\mu$ g/mL.







MassThe samples have been treated with trypsin for 1h at 57°C (Tryp\_CoV\_2) or trypsin for 1h at 57°C followedSpectrometry<br/>analysis:by PNGaseF for 1h at 37°C (Tryp\_PNGaseF\_CoV\_2). Comparison of samples provides knowledge of the<br/>glycosylation sites and sequence. Samples analyzed using Thermo Scientific™ Exploris™ 480 Mass<br/>Spectrometer.

N42(IT) and N54(AT) have been found to contain glycan structures.

Without PNGaseF

```
MGILPSPGMPALLSLVSLLSVLLMGCVAFRVQPTESIVRFPNITNLCPFGEVFNATRFAS60VYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQ120IAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDIS180TEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKK240STNLVKNKCVNFGHHHHHHH260260100100
```

Coverage: 214/260 82.31% own|Spike1|Spike-sRBD 319-514\_tyrPho-sig

#### With PNGaseF

```
MGILPSPGMP
ALLSLVSLLS
VLLMGCVAFR
VQPTESIVRF
PNITNLCPFG
EVFNATRFAS
60

VYAWNRKRIS
NCVADYSVLY
NSASFSTFKC
YGVSPTKLND
LCFTNVYADS
FVIRGDEVRQ
120

IAPGQTGKIA
DYNYKLPDDF
TGCVIAWNSN
NLDSKVGGNY
NYLYRLFRKS
NLKPFERDIS
180

TEIYQAGSTP
CNGVEGFNCY
FPLQSYGFQP
TNGVGYQPYR
VVVLSFELLH
APATVCGPKK
240

STNLVKNKCV
NFGHHHHHH
260
260
240
```

